This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

0861

REVIEW CRITERIA FOR NUCLEIC ACID AMPLIFICATION-BASED IN VITRO DIAGNOSTIC DEVICES FOR DIRECT DETECTION OF INFECTIOUS MICROORGANISMS

This is a flexible document representing the current major concerns and suggestions regarding in vitro diagnostic devices employing nucleic acid amplification and hybridization for direct detection of infectious microorganisms in clinical specimens. It is based on 1) current basic science, 2) clinical experience, 3) previous submissions by manufacturers to the FDA, and 4) the Safe Medical Devices Act of 1990 (SMDA) and FDA regulations in the Code of Federal Regulations (CFR). As advances are made in science and medicine, these review criteria will be re-evaluated and revised as necessary to accommodate new knowledge.

PURPOSE: This document provides general guidance to manufacturers about the information needed by the FDA to approve or clear in vitro diagnostic devices intended to detect microorganisms directly from human specimens using nucleic acid amplification and hybridization methods. The guidance supplements regulations in 21 CFR Parts 800-1299.

DEFINITION: The generic type device is intended for use in clinical laboratories as an in vitro diagnostic device for direct detection of microbial nucleic acids in clinical specimens. These qualitative assays are intended as an adjunct to culture (or other "gold standard", as appropriate).

PANEL: Microbiology (83)

BACKGROUND: Nucleic acid hybridization-based assays are currently used in clinical microbiology laboratories to identify cultured microorganisms or for direct detection of pathogens in clinical specimens. These assays are based on detecting unique nucleotide sequences found in the DNA or RNA of the target organism, whereas traditional identification methods have detected phenotypic characteristics (antigens, presence of enzymes using biochemical substrates, fatty acid analysis, etc). A nucleic acid probe, consisting of the complementary sequence of nucleotide bases to the target nucleic acid, binds to the target sequences. Probes are labeled with enzymes, chemiluminescent molecules, or other substances to produce a detectable signal when a hybrid is formed.[1]

Assay specificity is determined by the stringency or degree of binding between the target and probe nucleic acids. The degree of stringency is regulated by varying the conditions of the hybridization reaction. Depending on the target nucleic acid sequence selected, probes may be family-, genus-, species-, or strain-specific.

Assay sensitivity is affected by the minimum number of copies of

nucleic acid detectable by the probe and the signal-generating capabilities of its label. Most assays using hybridization methods only, require numerous copies of the homologous target nucleic acid (approximately 10^4 to 10^5 copies under ideal circumstances).[2] Greater numbers of copies are usually obtained by growing the organism on solid media, cell culture, or in broth prior to assay. Although these assays are a significant improvement in technology, hybridization assays have lacked sufficient sensitivity for direct detection of small numbers of organisms in specimens.

Recent advances in molecular technology have resulted in the development of assays capable of enzymatically amplifying low numbers of target nucleic acid (either in the target organism or the probe itself).[2] Amplification methods can increase the number of nucleic acid molecules available for hybridization, and offer the advantage of higher analytical sensitivity that could potentially translate into higher clinical sensitivity. Methods used to amplify nucleic acids include the polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription-based amplification systems (TAS), cycling probe reaction, strand displacement amplification, and QB replicase. Each method may also have several modifications (e.g., nested PCR).[3] Other techniques may amplify the intensity of the detectable signal of hybridization reactions.

Nucleic acid amplification methods offer the potential for timely identification of many causative agents of infectious disease heretofore requiring lengthy and/or difficult isolation and identification procedures. As with any new and evolving technology, numerous issues emerge in the review of molecular diagnostic methods. Manufacturers must address these issues and include the general information described below in their submissions. Additional information specific to the target organism will be discussed in supplements to the general guidance.

I. CLINICAL INDICATIONS/SIGNIFICANCE/UTILITY

Provide the following information about the device:

A. Description of the specific microorganism(s) detected.

B. Description of the disease/syndrome associated with the infection caused by the target microorganism.

C. Describe the epidemiology, prevalence rates, and groups at risk for the disease.

D. Is the organism a constituent of human normal flora?

E. A discussion of historical methods used to detect the organism including any other nucleic acid detection

approaches and a comparison (similarities and differences) of the new device to previously marketed devices. Describe the reference or "gold standard", if available, for detecting the organism in clinical samples.

F. Significance and clinical implications of false-positive and false-negative results, including consideration for variations in disease prevalence.

G. A discussion of the relevant medical/public health issues that may impact on the review.

II. DEVICE DESCRIPTION

Key issues in the review of a new device are the specific intended use (the organism detected and the indications for use), the specimen source tested, and the technology utilized. The following descriptive information must be included in a submission to adequately characterize the new in vitro device for detection of infectious microorganisms in clinical specimens with nucleic acid amplification methods. Appropriate peer-reviewed literature references that relate to the technology of the device must be submitted in addition to the descriptive information requested below to adequately describe the new in vitro device.

A. Intended Use

Describe the intended use of the device and provide the following information:

1. Description of the patient population to be tested using the device and the test setting.

2. Significance/clinical utility of the device. How will the device be used in a diagnostic algorithm? What other clinical/laboratory tests must be done in conjunction with the assay?

3. Rationale for using a nucleic acid amplification assay for the detection of the target organism and the clinical/diagnostic benefits compared to other methods.

4. Types of acceptable test specimens (matrix).

5. Specific organism(s) detected.

B. Detailed Principle of the Device Methodology

Provide a thorough explanation of all aspects of the test methodology including target amplification, hybridization (if applicable), and detection of amplification products. Provide information to substantiate application of the methodology to the detection of the specific etiologic agent; cite appropriate peer-reviewed literature references.

Include a complete description of the following items as appropriate:

1. Organism Target Nucleic Acid Sequence(s):

   Describe the organism target nucleic acid sequence and include the following information:

   a. Size (number of nucleotide bases); actual sequence of bases or a restriction endonuclease map; the specific location of the sequence on the genome, plasmid, or RNA; property encoded; number of sequence copies; and reference, if sequence has been published. Provide an explanation if this information is not known.

   b. Rationale for selecting the sequence(s) as the target.

   c. Justification for selecting a single or multiple target sequence(s).

   d. Degree of conservation of the organism target sequence. Is the target sequence family-, genus-, species-, or strain-specific?

   e. When the target sequence is plasmid DNA, what steps are taken to identify plasmid-free strains of the target organism or plasmid transfer to nontarget organisms? Provide appropriate limitation statements in the labeling.

2. Nucleic Acid Primers and Probes

   Characterize all primer and probe nucleic acid sequences used in the device by including the following information:

   a. Size, G+C content, type of primer or probe (DNA or RNA), restriction enzyme map, nucleotide base sequences, recognition site.

   b. Sequence accession number if registered with GenBank/EMBL data banks or other registration, licensure/patent information.

   c. If oligonucleotides are used, discuss their production, including instrumentation used, purification, and validation after synthesis.

   d. If cloned primers are used, describe the source and sequence of the nucleic acid and purification process.

e. Specify internal secondary structures, and complementarity of ends.

f. Detail function of multiple probes or primers.

g. Specify any conjugates that allow for capture or detection; describe the manner of attachment and the stability of attachment.

h. A summary of results of a database search to determine homology to known sequences of DNA.

i. If the probe was a component of a previous submission, reference the PMA or 510(k) number; provide all relevant information as described above in the current submission.

3. Specimen Preparation

a. Specify minimum volume of specimen type(s) required.

b. Describe reagents or other required materials and equipment used and their function in the specimen preparation process.

c. Verify that specimen processing reagents (or materials) are suitable for amplification, i.e., that they are free of microorganisms or amplifiable nucleic acid, and nucleic acid inhibitors.

4. Amplification and Other Enzymes

a. Describe the source and purpose of all enzymes.

b. Describe enzyme characteristics (thermostability, purity, etc.).

c. Describe equipment used and amplification conditions (time, temperature, number of cycles, etc.).

d. State anticipated amount of nucleic acid generated.

e. Describe handling of amplified products prior to hybridization/detection.

5. Controls/calibrators provided in the assay kit and the aspects of the procedure they control. The following controls or information must be provided:

a. One or more positive controls containing a

specific number of copies of the target nucleic acid sequence; the number of copies, determined individually for each target sequence, must be at or near the lowest claimed limit of detectable copies for one of the controls.[3]

b. A negative control containing nontarget nucleic acid sequences.

c. Controls must be in the same matrix as specimens indicated for testing. Provide justification for selection of control materials including matrix choice.

d. Recommendations for other types of controls (reagent, coamplification or spike-back procedures, cell lysis, and/or extraction)[4] or justification for excluding such controls. If controls are excluded, labeling must include limitations statements about potential false-negative results.

e. Provide acceptable values for controls and justification for values selected.

6. Additional reagents provided in the kit or recommended for use and their function in the assay.

7. Determination of the cut-off value(s) or endpoint(s) for the assay. Required validation data is described below in Section III.

8. Safety aspects for performing the assay. Specify at what procedural step the testing material is non-infectious.

9. Software elements and dedicated instrumentation responsible for specimen handling, amplification, hybridization, or detection, and/or used to determine/calculate assay results. See requirements for Moderate Level of concern in Reviewer Guidance for Computer Controlled Medical Devices Undergoing 510(k) Review (available from the Division of Small Manufacturers Assistance). Furnish the following for dedicated instrumentation and software elements:

a. Reference premarket notification [510(k)] submission number for any non-dedicated instrument.

b. Algorithms used to calculate results in either dedicated or non-dedicated instruments.

c. Mathematical curve-fitting method(s) used when results are calculated by instrument-related software.

C. Specimen collection/transport devices.

Detail the method(s) that must be used to collect optimal specimens for testing.

1. For specific collection or transport devices recommended or included in test kit, specify ways users should assure that specimens were collected and transported appropriately.

2. Specify the types and volume (if applicable) of all specimens acceptable for testing with the device. Discuss the effects of testing inadequate or inappropriate specimens.

3. List the appropriate specimen transport conditions (e.g., time, temperature, etc.) for each type of specimen. List and discuss the effect(s) of inappropriate transport.

4. Describe recommended storage time and temperature.

D. Merits and Limitations of the Method

Discuss the merits, limitations, advantages, disadvantages of the device compared to other available test methodologies. Concerns with nucleic acid amplification methods are: adequate sample preparation, presence of sample-related inhibitors, risks of sample contamination, adequate quality control, relationship of a positive/negative result to disease presence/absence, and ability of laboratories to reproducibly detect a consistent level of organism load.

III. SPECIFIC PERFORMANCE CHARACTERISTICS

The FDA requests different types and amounts of data and statistical analyses to market in-vitro diagnostic devices. The amount and types of data requested depend on the intended use and the technological characteristics of the new device. The data and statistical evaluation must be sufficient to demonstrate the safety and effectiveness of the device for all claimed specimen types, assay reproducibility, and substantial equivalence to a previously marketed device with the same intended use and technology, or to the generally accepted gold standard (e.g., culture). When the gold standard is flawed, or there is none, clinical data from well-controlled trials will be required to support performance characteristics.

Provide complete procedures for all studies. All testing to

establish the performance characteristics of the device must be performed with a final form of the device according to instructions provided in the product labeling. Present test data with analyses and conclusions; include explanations for unexpected results and any additional testing performed. When appropriate, charts (scattergrams, histograms, receiver operator curves (ROC), etc.) may be used as part of the analyses and conclusions. Raw laboratory data with quality control results may be requested.

A. Analytical Laboratory Studies (Phase I and II)

Specific parameters of importance to the operation of the device must be supported by data determined with the device prior to testing in outside laboratories. This testing should be done in-house or at a designated laboratory facility as part of the test development phases. The following types of studies must be performed to determine operational parameters and assess the specific performance of the device:

1. Validation of Cut-off and/or Calibration Curve.

   a. Evaluate specimens from patients clinically diagnosed as positive for the disease and specimens from asymptomatic, healthy individuals (or patients with other infections/diseases/syndromes). The number of specimens in each category should be statistically significant and include specimens in all sample matrices used in the assay. Document and reference the diagnostic criteria for characterizing patients.

   b. Describe the rationale for determining the assay cut-offs. Furnish descriptive information and laboratory data to show how the cut-off distinguishes between positive and negative samples.

   c. Define the statistical method used to determine the cut-off.

   d. Present a ROC analysis of cut-off selection and other graphical representations as appropriate.

   e. Define the basis for the equivocal zone (if applicable).

   f. Demonstrate that amplified products in ten of the positive specimens contain the anticipated theoretical number of nucleic acid bases.

2. Limits of Detection

   a. Establish the limits of detection or endpoint of

the assay by determining the minimum number of copies of nucleic acid detectable.

(1) In addition to testing known numbers of copies, use independent dilutions of specimens with known numbers of organisms; test each in triplicate.

(2) Relate the number of detectable copies to colony forming units (CFU)/ml, plaque-forming units (PFU)/mL, or other method when organisms are non-culturable (e.g., approximate numbers of organisms visualized microscopically).

(3) Use the same specimen matrix and diluents used for clinical specimens to perform studies. If multiple sample matrices are used in the assay, endpoints must be determined for each type.

b. Provide data to support the ability to detect a wide variety of isolates representing documented organism diversity (e.g., geographic areas, serovars or serotype, drug resistance, etc.)

3. Validation of Assay Specificity

Demonstrate the device's specificity by performing nucleic acid amplification studies on well-characterized isolates:

a. Strains must include closely related genera, other pathogenic and commensal flora found in the same anatomical sites as those tested by the device, and unrelated genera.

b. List the genus and species, number of each tested, and reference numbers if applicable.

c. State the inoculum used and documentation of method used in the studies (10^8 cfu/ml suggested for most bacteria).

4. Interference Studies

Provide data to demonstrate that potentially interfering substances encountered in specific specimen type(s) do not affect assay results. An appropriate limitation must be included in the package insert whenever interference is demonstrated or cannot be discounted. The following are examples of substances that may interfere with nucleic acid amplification

methods:

a. Endogenous substances likely to be present in patients' specimens (e.g., blood, mucus, pus, etc.).

b. Possible exogenous substances present in specimens or used in the specimen collection process (e.g., medications, local antiseptics or anesthetics, spermicides, specimen preservatives, etc.).

c. Nonspecific inhibitors. What is the incidence of unexplainable, nonspecific inhibition?

6. Validation of Decontamination Methods

If manufacturers include reagents (e.g., uracil-DNA glycosylase, formamide, methoxysoralen, restriction enzymes, etc.) or recommend procedures (e.g., irradiation) for inactivating contaminating nucleic acids, provide the following information:

a. Data to support performance of the reagent or procedure.

b. Determine the sensitivity and specificity of the reagent/procedure by performing studies using varying dilutions of treated and untreated amplified or (amplifiable) nucleic acids.

7. Provide data to validate recommended transport and storage conditions. Define the optimal conditions based on real-time specimen storage stability studies. Evaluate transport and storage effects for both false positivity and negativity.

8. Provide data to demonstrate that testing materials are non-infectious at a specified procedural step.

9. Precision

The National Committee for Clinical Laboratory Standards (NCCLS) recommends an analysis of variance experiment that permits estimation of within-run and total standard deviations (SD).[5] See the NCCLS Guideline for recommended data collection formats and calculations. Perform separate calculations for each specimen tested for within-run and total precision.

Test ten clinical or simulated specimens with varying numbers of organisms; include 2 specimens at the lower limit of detection plus the controls supplied with device in triplicate on three different days at three

laboratory sites (10 specimens plus controls tested X 3 X 3 days X 3 sites). One testing site may be in-house. If additional matrices may be used, include three additional samples (non-reactive, low positive, and positive) for each matrix.

For calculated endpoint tests, present coefficients of variation for each set of values for with-in run and total precision, using units defined in the test procedure.

For single endpoint assays, provide percentage of results negative, borderline/equivocal, or positive for each set of tests.

If dedicated instrumentation is used in specimen handling, or reading and interpreting results, use a different instrument (different serial number) at each site. If non-dedicated instruments are used, state specifications of instrument(s) used at each site.

B. Clinical Studies.

Because nucleic acid amplification methods can potentially replace culture methods for rapid diagnosis of infectious diseases, the device must detect the presence of the target organism. Appropriate clinical studies may be necessary to show the relationship of results to the patient's clinical condition (e.g., pre-disease state, disease presence, carrier state, asymptomatic or symptomatic infection, presence of viable or non-viable organisms, etc.).

Clinical testing should be performed by independent laboratories not affiliated with the manufacturer, and in different geographic locations; testing sites must be representative of the settings indicated for actual testing. Manufacturers must indicate the steps taken to assure that laboratories performing tests for the studies use optimal procedures for the "gold standard". Identify testing sites by institutional name and address; include the name, title, and phone number of the responsible investigator(s) at each site.

Compare the new device to culture (or alternate "gold standard" when culture methods are flawed or organism is non-culturable). All testing must be performed concurrently using testing, storage, collection procedures, and interpretative criteria specified in the package inserts. Adequate numbers of different sample matrices should be included as appropriate. Statistical analysis must include point estimates and 95% confidence limits.

Provide the study protocol used at each site. The protocol must indicate specimen selection criteria, specimen handling

and storage procedures, the culture method or other "gold standard" used, identification procedures, blinding procedures, the process for review and analysis of results.

1. Describe the study protocol design in detail, including the following information:

    a. All testing must use fresh clinical specimens routinely received in the laboratory for culture (or other method of detection/identification) of the target organism.

    b. Laboratory findings must be documented for each specimen and include, as appropriate, specimen source, results of other cultures from the same patient, semi-quantitation of culture results, and microscopic results.

    c. The new device must be used during the study in accordance with its intended use and specimens tested must represent appropriate populations for use of the test. In addition, specimens from patients with diseases or conditions with similar symptomology, and/or specimens from healthy individuals must be included.

    d. Provide a scheme for resolving results that are discrepant with culture; describe how these results will be handled in statistical analysis. Strategies may include repeat testing of the original sample, repeat testing using alternate primers or extraction procedures, testing additional samples from patients, documentation of amplified nucleic acid sequences by electrophoretic gels or blotting techniques, documentation of the patient's history, and/or amplification of isolates on culture media. Include all data for repeat tests and alternate procedures.

2. Testing must be performed on an adequate number of positive and negative clinical specimens, from appropriate patient populations, sufficient to demonstrate, with confidence, the safety and effectiveness of the device for its intended use.

3. Describe the patient selection criteria and any exclusion criteria. Provide demographic and diagnostic information about the study population.

4. For final data analysis, calculate sensitivity and specificity along with 95% confidence limits for each site, specimen type, and patient population or diagnostic group; provide overall statistics for the

device, with a significance analysis of difference to the gold standard method.

5. Provide quality control results for each day of testing. Document QC done to monitor culture procedures and/or other methods used.

IV. LABELING CONSIDERATIONS

The following are additional details for some of the points in the statute [502(f)(1)] and regulations [21 CFR Section 809.10(b)].

A. Intended Use Statement

The statement should be a concise description of essential information about the product. An example follows:

The ____ Assay is for the qualitative detection of nucleic acids of (name of target organisms) in (specimen types) using (methodology) as an aid in the diagnosis of (infectious disease).

B. Indications for Use

Indications for use of the device should describe any special applications of the device or specific contraindications or indications for use not addressed in the Intended Use Statement."

These conditions for use may be further detailed in either the Summary and Explanation, Limitations, and/or Performance Characteristics Section of the package insert.

C. Specimen Collection and Handling

1. State the type of specimen to be collected, and the types of collection devices which may be used. Specify incorrect collection procedures.

2. State the conditions for patient preparation, e.g., timing of collection, order of collection, etc.

3. Provide adequate directions for sample collection and/or references for appropriate collection procedures, e.g., textbooks, journals, etc.

4. Identify interfering substances or conditions.

5. Provide instructions for transport to the laboratory or testing. Specify inappropriate transport conditions.

6. State the specimen storage conditions and stability periods.

7. Provide a mechanism for the laboratory to assure collection and transport requirements have been followed.

C. Warnings and Precautions

List the special precautions required to avoid or minimize contamination with carryover nucleic acids from personnel or environmental sources. Precautions may include the following steps if appropriate:

1. Performing specimen preparation (nucleic acid extraction), preamplification preparation and set- up, amplification, and detection phases of the assay in separate areas of the laboratory or preferably in different rooms.

2. Using dedicated pipettors and other equipment for each phase of the assay.

3. Using positive displacement pipettors or pipette tips with hydrophobic plugs.

4. Minimizing aerosol formation and preferably using a biological cabinet for preparation phases.

5. Wearing laboratory coats and gloves during assay including frequent changing of gloves.

6. Place positive controls following patient samples in test run.

7. Personnel performing molecular assays must have specialized experience/training.

8. Use only the supplied or disposable laboratory ware.

9. Decontaminate work surfaces and pipettors with 10% household bleach.

10. Disposal and decontamination instructions for reaction mixtures and waste upon test completion.

11. Instructions for handling hazardous reagents or specimens.

C. Limitations of the Procedure

List the important limitations and known contraindications of the assay with available references. Examples of limitations are included in the following statements:

1. Nucleic acid amplification results must be used in conjunction with other clinical or laboratory

information for diagnosis of infectious diseases.

2. Test only the specimens indicated. Testing of other specimen types may result in false negative or positive results.

3. False-negative results may result from improperly collected, transported, or handled specimens, procedural errors, amplification inhibitors in the specimen, or inadequate numbers of organisms for amplification. Contamination from carryover nucleic acids may result in false-positive determinations.

4. Specimen adequacy cannot be assessed.

5. Therapeutic outcomes cannot be determined as nucleic acid target may persist independent of organism viability following appropriate therapy.

6. For genus-specific probes, multiple species of the genus present in the specimen cannot be distinguished.

D. Quality Control

Provide the following information:

1. Recommendations for testing frequency and placement of assay controls/calibrators.

2. Directions for interpretation of the results of quality control samples.

3. A statement similar to the following: "If controls do not behave as expected, results are invalid and patient results should not be reported."

4. Troubleshooting instructions when controls are out-of-range.

5. Provide alternate QC recommendations to control all aspects of the procedure.

NOTE: Quality control requirements may be affected by CLIA regulations.

E. Expected Values

1. Indicate the expected prevalence of the disease in diverse patient populations.

2. Indicate that prevalence may vary depending on geographical location, age, gender of population studied, type of test employed, specimen collection and handling procedures, clinical and epidemiological

history of individual patients, etc.

F. Results

1. Include explanations for interpretation of results. Provide cut-off levels as appropriate.

2. Include terminology recommended for reporting patients' results. The methods for reporting non-reactive results could be "no nucleic acids to (name of organism) detected." The term "negative", used alone, should be avoided. Use a similar approach for results above the cut off.

3. Provide recommended follow-up action for equivocal/borderline results if appropriate (e.g., "If result is equivocal on repeat testing, obtain a new specimen and reassay").

G. Performance Characteristics

Provide summaries of the performance data for the assay, e.g., clinical sensitivity and specificity compared to clinical diagnosis and culture. Positive and negative predictive values should be based on specific populations sampled for each disease syndrome. State the prevalence at each testing site and show the effect of prevalence on positive and negative predictive values in different test populations. Include the following items:

1. Present cross-reactivity studies in a tabular form; specify all positive and borderline/equivocal/ indeterminate results.

2. Present limits of detection for all species or all strain variants as appropriate. Include data for number of copies and number of organisms (CFU, PFU, etc.) detected.

3. Summarize within-run and total precision.

4. Summarize reproducibility data.

5. Present data from comparison/clinical studies, using separate categories for different patient groups and specimen sources (e.g., symptomatic vs. asymptomatic). Clearly display all borderline/equivocal/indeterminate results. Discrepancies between test and culture results (or other gold standard or clinical diagnosis) may be discussed. Present final sensitivity, specificity, positive and negative predictive values, based on the total number of true positive and negative specimens determined by culture results (or other gold standard), clinical diagnosis based on other clinical/-

laboratory information, or a combination. Define methods for determining true positives and negatives. Include 95% confidence limits along with point estimates of the above parameters.

V. References

1. Tenover F. Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin Microbiol Rev 1988;1:82-101.

2. Persing DH. In vitro nucleic acid amplification techniques, p51-87. In DH Persing, TF Smith, FC Tenover and TJ White (ed), *Diagnostic Molecular Microbiology, Principles and Applications*. American Society for Microbiology, 1993, Washington DC.

3. Wolcott M. Advances in nucleic acid-based detection methods. Clin Microbiol Rev 1992;5:370-386.

4. Cone R, Hobson A, Huang M. Coamplified positive control detects inhibition of PCR. J Clin Microbiol 1992;30:3185-89.

5. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices, tentative guideline. 1991. Order Code EP5-T2.